Synthesis and Biological Activity Studies of Some Simple and Fused Nitrogenous Heterocycles

Synopsis

of the Ph.D. Thesis to be submitted to the

MANGALORE UNIVERSITY

For the Award of the Degree of

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

By

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DEPARTMENT OF CHEMISTRY

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PRE-SYNOPSIS OF THE THESIS

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Title of the Thesis: Synthesis and Biological Activity Studies of Some Simple and Fused Nitrogenous Heterocycles

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SYNOPSIS

Indeed, heterocyclic compounds play a crucial role in organic chemistry, and their diverse biological activities make them significant in medicinal chemistry and drug development too.

The importance of heterocyclic compounds, especially those with five or six-membered rings, lies in their prevalence in nature and their wide range of biological activities[1,2]. Many naturally occurring compounds with important biological functions are heterocyclic compounds. Examples include nucleic acids (DNA and RNA), which contain heterocyclic bases, and various vitamins, hormones, and alkaloids[3]. A significant number of pharmaceuticals and drugs are based on heterocyclic structures[4]. Heterocyclic compounds often exhibit strong interactions with biological macromolecules like proteins and this can be crucial for the design of drugs that target specific proteins or enzymes in the body. Heterocyclic compounds offer a vast array of structural diversity, allowing medicinal chemists to explore different molecular architectures in the search for bioactive compounds. Many heterocyclic compounds have shown promising antimicrobial and anticancer activities. The presence of nitrogen or other heteroatoms in the ring can influence the reactivity and interactions with biological targets. Moreover, heterocyclic compounds are also used in the development of herbicides and pesticides due to their ability to interfere with specific biological processes in plants or insects.

This is particularly true for compounds with five membered heterocycles, which are the building blocks of many compounds with a diverse variety of interesting

biological functions. In the long decades of organic synthesis, chemists are still interested in heterocyclic molecules comprising nitrogen, oxygen, and sulphur[4] due to their unique structures which served as a framework in the design of novel compounds with applications in the areas of medicinal, material sciences and agrochemical chemistry[5,6].

Several simple heterocyclic compounds namely Pyrazoles. Thiazolidinones, Benzimidazoles, Imidazopyridines, Pyridines are known to possess interesting biological properties.

Pyrazole derivatives exhibit a variety of biological activities, making them interesting compounds for pharmaceutical research[8]. The biological activities of pyrazole derivatives are often attributed to their ability to interact with specific biological targets, such as enzymes or receptors. Pyrazoles and its derivatives gained importance in recent years due to their chemotherapeutical value. Many pyrazole derivatives are found to be potent anthelmintic[9], anti-infective[10], anti-inflammatory[11], anticancer[12], herbicidal[13], analgesic[14], antimicrobial[15] and anti-oxidant[16] agents.

Thiazolidinones are a class of organic compounds have diverse pharmacological activities and are of interest in medicinal chemistry. One of the well-known applications of thiazolidinones is in the field of medicine, particularly as antidiabetic agents. These compounds have been investigated for their potential biological activities, especially in the context of medicinal chemistry. Thiazolidinones have been explored for their anticancer[17], antibacterial[18], antifungal[19], anti-inflammatory[20,21] and antiviral[22] properties.

Imidazopyridines are a class of organic compounds that have diverse applications in medicinal chemistry and drug development due to their interesting pharmacological properties. Some imidazopyridine derivatives have been studied for their effects on the central nervous system and are being explored as potential candidates for the treatment of neurological disorders. Imidazopyridines serve as a scaffold for the development of new pharmaceutical compounds. Structural modifications to the imidazopyridine core can lead to variations in biological activity and pharmacokinetic properties, making them valuable in the design of novel drugs. Certain imidazopyridines exhibit a range of biological activities, including anti-inflammatory[23], antiviral[24], anticandidal[25], anti-fungal[26], anticancer[27], anti-tumour[28], anti-hypertencive[29] and hypnotic activities[30].

Benzimidazole is a fused system of imidazole and benzene and their derivatives exhibit diverse biological activities, making them crucial in drug development. Some derivatives have demonstrated antimicrobial properties, serving as anthelmintic drugs (*e.g.,* albendazole and mebendazole) or antifungal agents (*e.g.,* thiabendazole). Benzimidazole derivatives represent a versatile class of compounds with wide-range of applications. Their unique chemical structure and diverse properties make them indispensable in the development of pharmaceuticals, materials, and other advanced technologies. Benzimidazole derivatives possess broad spectrum of pharmacological activities such as antimicrobial[31], antiulcer[32], anti-inflammatory[33], anticonvulsant[34], anticancer[35], and cholinesterase inhibition[36] activities.

The structure of pyridine imparts unique chemical and physical properties to its derivatives, making them widely used in various fields, including medicinal

chemistry. Pyridine derivatives exhibit diverse biological activities, and many of them have been developed into pharmaceuticals due to their potential therapeutic effects. The pyridine derivatives possess biological activities such as anti-bacterial[37], antimicrobial[38], anti-inflammatory[39], antioxidant[40], analgesic[41], antiviral[42], anticonvulsant[43], and anticancer[44] *etc.*

In view of these observations, it is therefore contemplated to synthesize some pyrazole endowed thiazolidinones, imidazopyridines, benzimidazoles, and pyridine derivatives and to screen them for their biological activities. Hence, the synthesis of new compounds belonging to such heterocyclic systems is thought to be important. The present study concerns with developing protocols for the synthesis of above-mentioned heterocycles carrying different substituents and study their antibacterial, antidiabetic, cytotoxicity, and antioxidant activities.

The thesis entitled, “Synthesis and biological activity studies of some pyrazoles endowed heterocyclic compounds” has been divided into six chapters.

Chapter 1

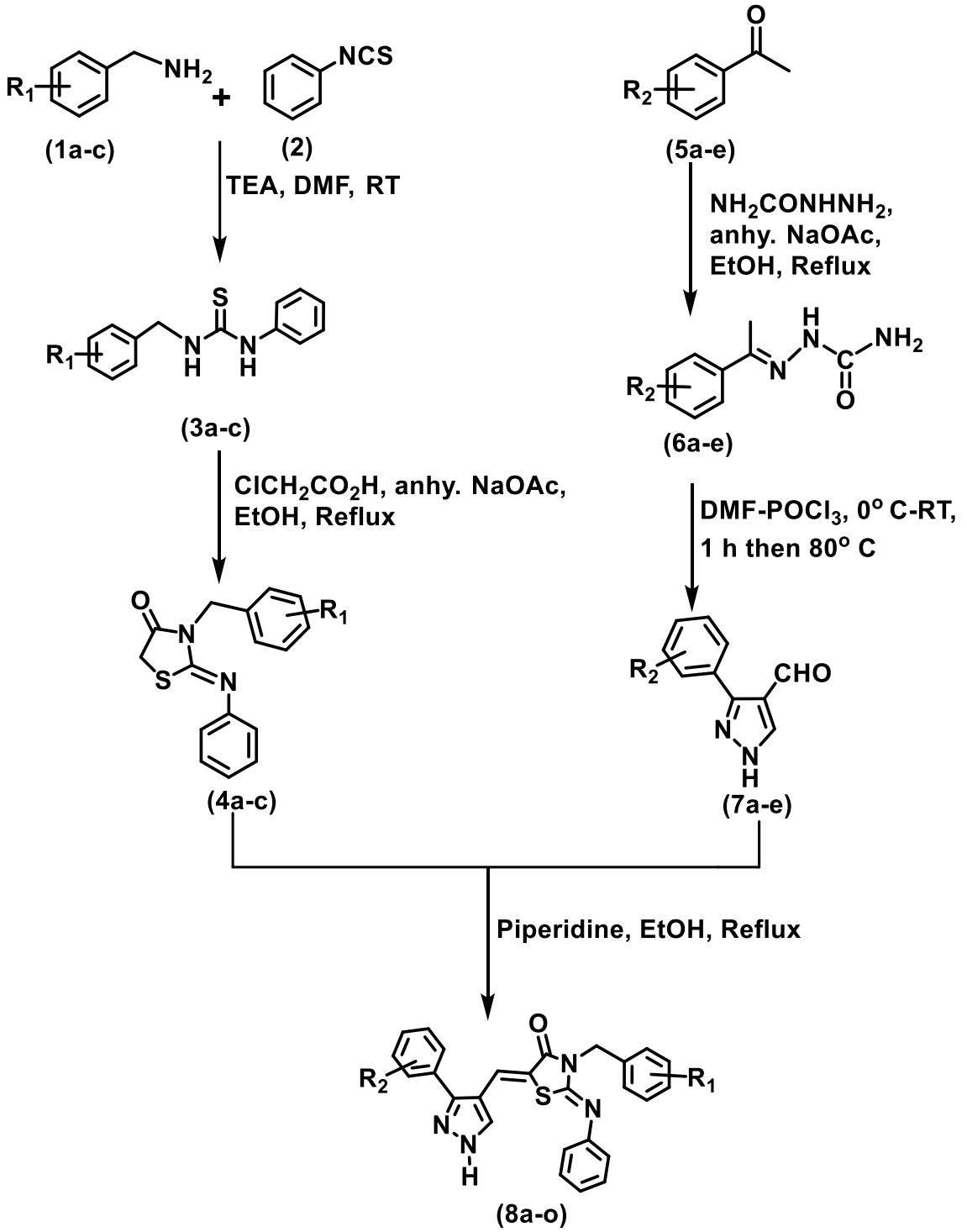
A review on synthesis and biological evaluation of some simple and fused nitrogenous heterocycles

This chapter serves as an introduction and briefly overviews the synthesis, reactions, applications, and biological activities of five and six membered simple and fused heterocycles. It is based on works published in the chemical literature up to January 2024.

Chapter 2

Synthesis and biological activity studies of fluorinated pyrazole endowed thiazolidinone derivatives

This chapter describes the synthesis, characterization and biological activity studies of some novel fluorinated pyrazole endowed thiazolidinone derivatives. Firstly, different fluorinated benzyl amines **(1a-c)** were reacted with phenyl isothiocyanate **(2)** in DMF medium in the presence of TEA as a catalyst at room temperature to obtain 1-(fluorinated benzyl)-3-phenylthioureas **(3a-c)**. These upon heterocyclization with chloroacetic acid in the presence of sodium acetate in ethanol medium produced fluorinated 2,3-disubstituted thiazolidin-4-ones **(4a-c)**. Secondly, different pyrazole aldehydes **(7a-e)** are obtained by treating different acetophenones **(5a-e)** with semicarbazide in the presence of sodium acetate in ethanol medium to obtain corresponding 1-[1-aryl-ethylidine]semicarbazides **(6a-e)**. These were then subjected to Vilsmeier-Hack formylation reaction with DMF-$POCl_3$ to obtain pyrazole aldehydes **(7a-e)**. Finally, target compounds *i.e.,* fluorinated pyrazole endowed 2,3-disubstituted thiazolidinones **(8a-o)** were synthesized by condensing fluorinated 2,3-disubstituted thiazolidin-4-ones **(4a-c)** with different pyrazole aldehydes **(7a-c)** using piperidine as catalyst in ethanol medium **(Scheme 1)**. All compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. These compounds were screened for their α-amylase inhibition and anti-oxidant activity. Furthermore, docking studies of the active compounds were performed to explore the possible binding interactions to understand the mode of action. The results of these studies are described in this chapter.



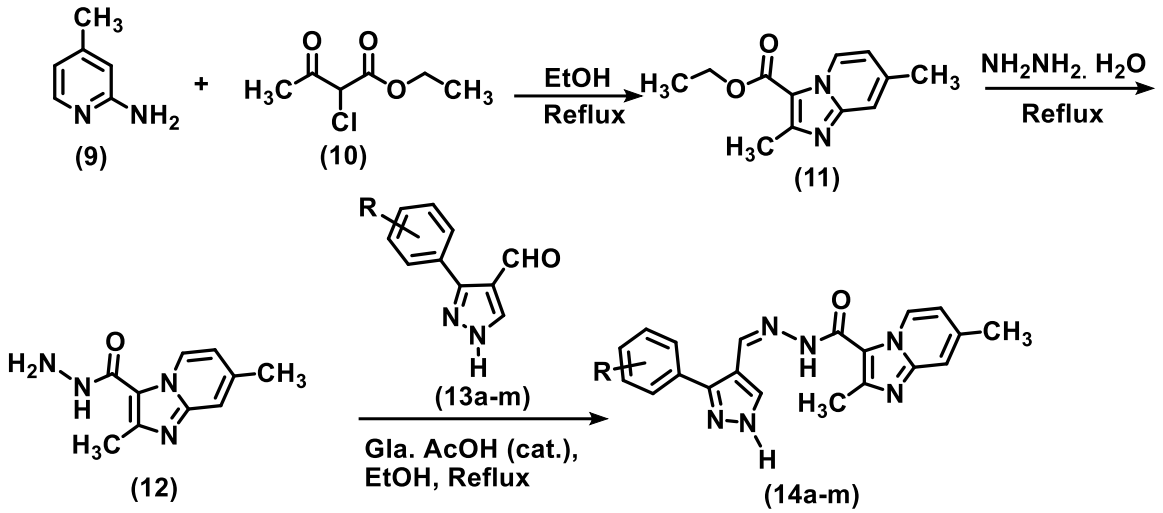
R_1 = 2-F, 3-F, 2,4-F_2; R_2 = 3,5-F_2, 2,4-$(OCH_3)_2$, 3,4-$(OCH_3)_2$, 4-(2-fluorobenzyloxy), 3-OCH_3-4-(2-fluorobenzyloxy).

Scheme 1: Synthesis of fluorinated pyrazoles endowed thiazolidinone derivatives **(8a-o)**

Chapter 3

Synthesis and biological evaluation of pyrazole embedded imidazopyridine derivatives

This chapter deals with the synthesis, characterization and antidiabetic activity studies of some pyrazole embedded imidazopyridine derivatives. Firstly, various aryl substituted-1*H*-pyrazole-4-carboxaldehydes **(13a-m)** were prepared from the corresponding acetophenones by two step reaction. Secondly, 2-amino-4-methyl pyridine **(9)** was converted to ethyl 2,7-dimethylindolizine-3-carboxylate **(11)** by heating with ethyl 2-chloro-3-oxobutanoate in absolute ethanol. This compound was then converted to corresponding hydrazide **(12)** upon reaction with hydrazine hydrate in ethanolic medium. Then final compounds, pyrazole embedded imidazopyridine derivatives **(14a-m)** were synthesized by condensing substituted-1*H*-pyrazole-4-carboxaldehydes **(13a-m)** with ethyl 2,7-dimethylindolizine-hydrazide **(12)** in ethanol with the aid of catalytical amount of glacial acetic acid **(Scheme 2)**. All compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. These derivatives were evaluated for their reverse inhibitory action on α-glucosidase and α-amylase and also the *in silico* studies. The results of these studies are described in this chapter.



R = 4-F, 4-Cl, 3-Br, 4-CH_3, 4-OCH_3, 2,4-F_2, 3,5-F_2, 2,4-Cl_2, 4-OH-3-OCH_3, 2,4-$(OCH_3)_2$, 3,4-$(OCH_3)_2$, 4-(2-fluorobenzyloxy), 3-OCH_3-4-(2-fluorobenzyloxy).

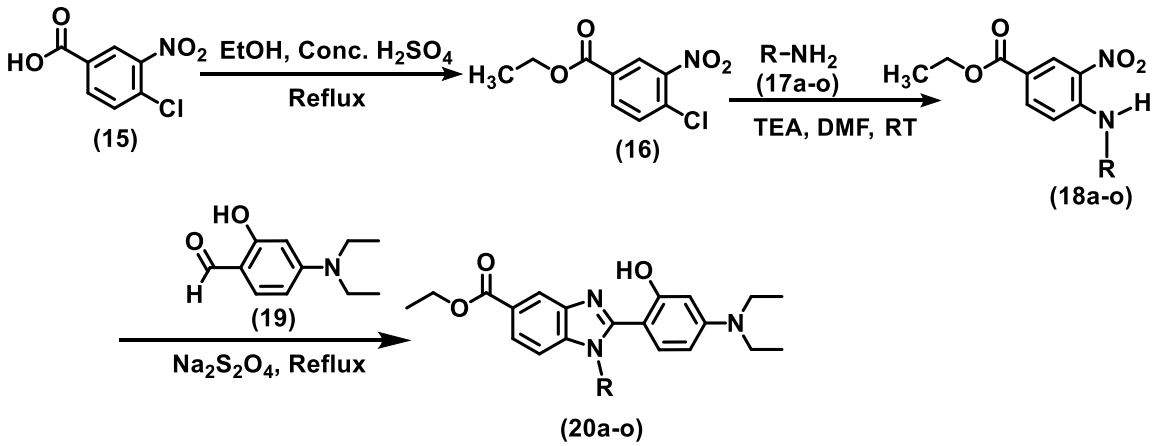
Scheme 2: Synthesis of pyrazole embedded imidazopyridines **(14a-m)**

Chapter 4

Synthesis, structural and anticancer studies of novel of 1,2-disubstitited benzimidazole-5-carboxylates

This chapter describes the synthesis, structural, anticancer and molecular docking studies of novel of 1,2-disubstitited benzimidazole-5-carboxylates **(20a-o)**. Firstly, 4-chloro-3-nitrobenzoic acid **(15)** was esterifies by refluxing in absolute EtOH in the presence of catalytic amount conc. H_2SO_4 to afford ethyl 4-chloro-3-nitrobenzoate **(16)**, This upon nucleophilic substitution reaction with different aliphatic/aromatic amines **(17a-o)** in the presence of TEA at room temperature yielded corresponding amino substituted ester **(18a-o)**. These were then subjected to one-pot nitro reductive cyclization reaction with 4-(diethylamino)-2-hydroxybenzaldehyde **(19)** in the presence of sodium dithionite as a reducing agent in DMSO yielded final compounds *i.e.,* 1,2-disubstitited

benzimidazole-5-carboxylates **(20a-o)** as shown in **Scheme 3**. The obtained products were purified by recrystallization in DMF. The formation of these products were confirmed by ^{1}H NMR, ^{13}C NMR, MS, FT-IR, single crystal XRD and elemental analyses. *In vitro* anticancer activities for the compounds assessed through a cell-based assay against Human lung cancer A549 and breast cancer MCF–7 cell lines. The results of these studies are described in this chapter.



Scheme 3: Synthesis of ethyl 1-substituted-2-(4-(diethylamino)-2-hydroxyphenyl)-benzimidazole-5-carboxylates **(20a-o)**

Chapter 5

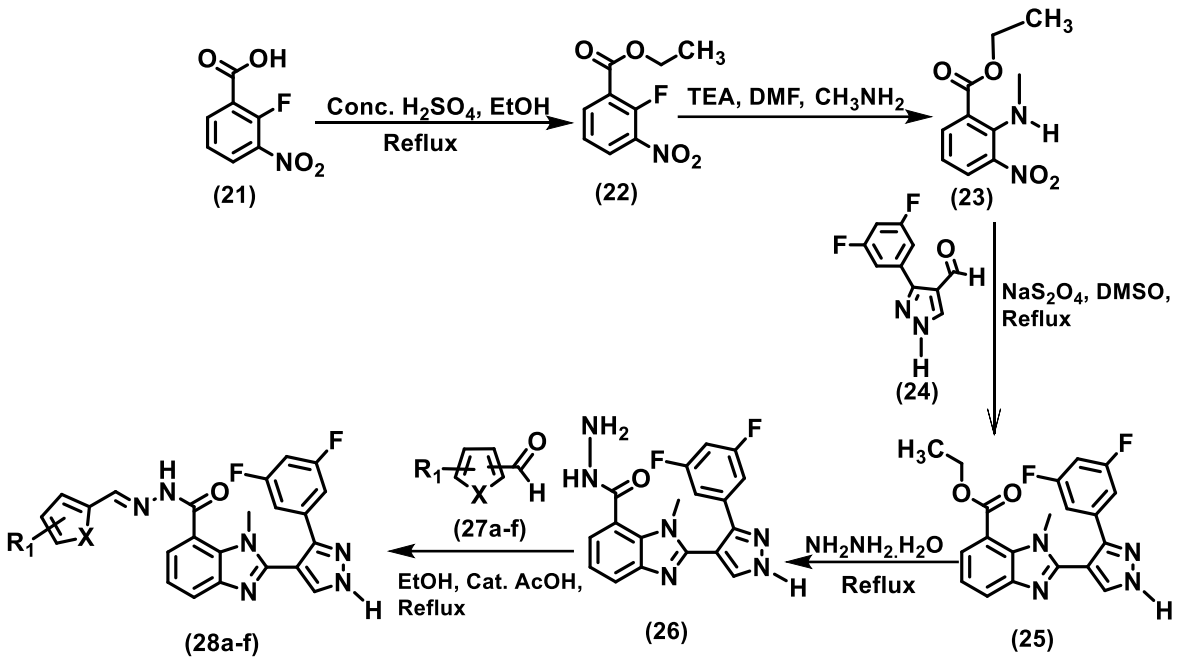
Synthesis and antimicrobial evaluation of pyrazole clubbed benzimidazole hydrazones

This chapter deals with the synthesis and characterization of pyrazole clubbed benzimidazole hydrazones, followed by their biological evaluation as anti-microbial agents. For the synthesis of first series of pyrazole clubbed benzimidazole hydrazones **(28a-f)**, the esterification of 2-fluoro-3-nitrobenzoic acid **(21)** with ethanol was performed under reflux condition using a catalytic amount of conc. H_2SO_4 to obtain ethyl-2-fluoro-3-nitrobenzoate **(22)**. This upon nucleophilic

substitution of fluorine with methylamine at room temperature in presence of TEA in DMF offered the ethyl 2-(methylamino)-3-nitrobenzoate **(23)**. Further, the one-pot cyclization reaction of compound ethyl 2-(methylamino)-3-nitrobenzoate **(23)** with 3-(3,5-difluorophenyl)-1*H*-pyrazole-4-carbaldehyde **(24)** in DMSO using sodium dithionite as a reducing agent produced the ethyl 2-(3-(3,5-difluorophenyl)-1*H*-pyrazol-4-yl)-1-methyl-1H-benzo[d]imidazole-7-carboxylate **(25)**. This ester was then converted to corresponding acyl hydrazide **(26)** upon reaction with hydrazine hydrate in ethanolic medium. Then to accomplish the first series of target compounds **(28a-f)**, pyrazole endowed benzimidazole hydrazide **(26)** was condensed with various heterocyclic aldehydes **(27a-f)** in ethanol with the aid of catalytical amount of glacial acetic acid (**Scheme 4.1**).

For the synthesis of second series of target compounds **(36a-f)**, 4-chloro-3-nitrobenzoic acid **(29)** was esterified in ethanol under reflux condition using catalytic quantity of concentrated H_2SO_4 to get the ethyl-4-chloro-3-nitrobenzoate **(30).** Further, ethyl 4-(methylamino)-3-nitrobenzoate **(31)** was obtained in a nucleophilic substitution reaction of chlorine of the intermediate ester **(30)** with methylamine at room temperature in the presence of TEA in DMF medium. The ethyl 2-(3-(3,5-difluorophenyl)-4,5-dihydro-1*H*-pyrazol-4-yl)-1-methyl-1*H*-benzo[*d*]-imidazole-5-carboxylate **(24)** was prepared by one-pot nitro reductive cyclization reaction between compound **31** and 3-(3,5-difluorophenyl)-1*H*-pyrazole-4-carbaldehyde **(24)** in DMSO using sodium dithionite as reducing agent. This ester was treated with hydrazine hydrate in an ethanolic medium to get corresponding hydrazide **(33).** Then, the second series of final compounds (**34a-f)** were synthesized by condensing hydrazide **33** with various heterocyclic aldehydes (**27a-**

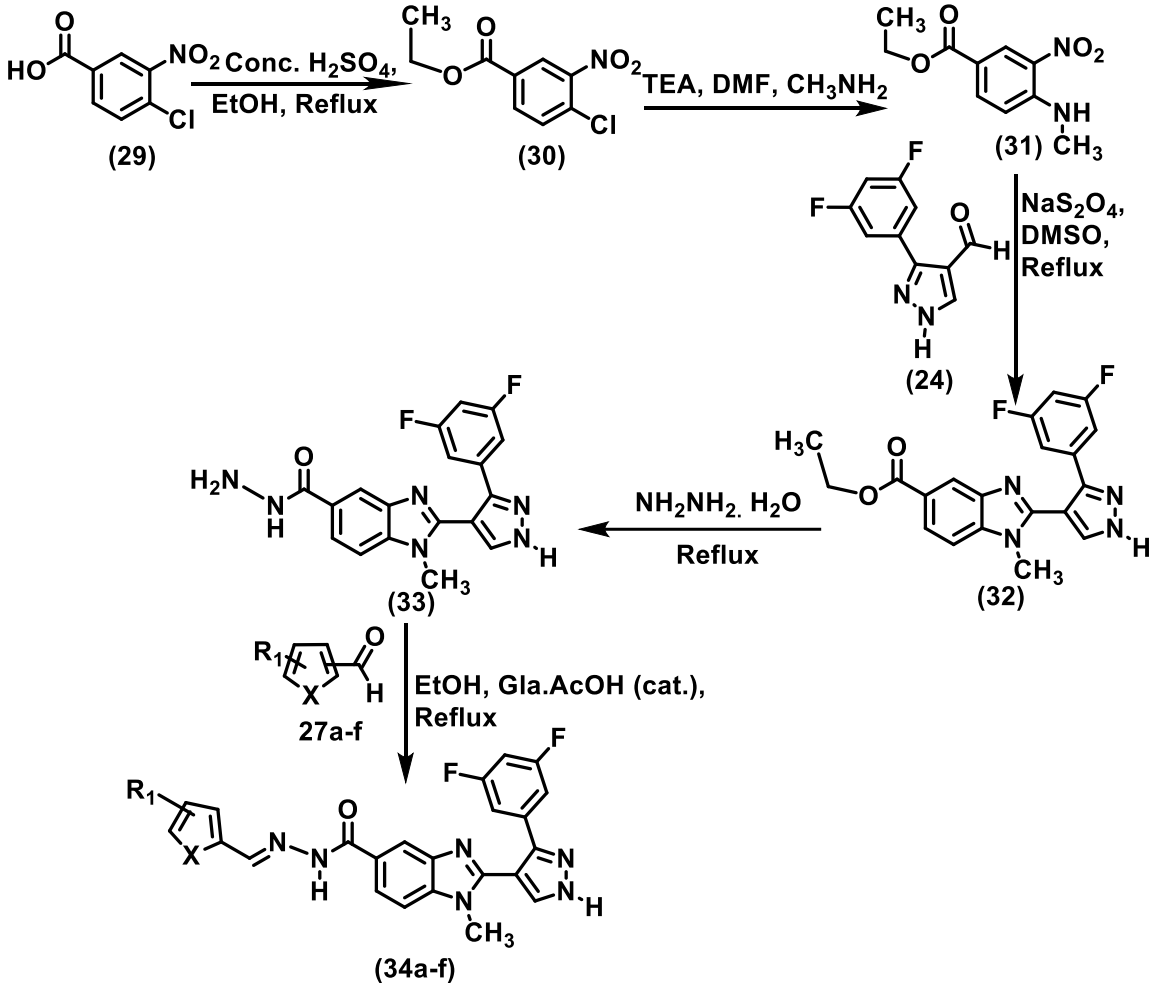
f) in ethanol with the aid of catalytic amount of glacial acetic acid as shown in **Scheme 4.2**



X = O; R_1 = H, 5-CH_3, 5-NO_2, 5-Cl

X = S; R_1 = H, 3-CH_3

Scheme 4.1: Synthesis of first series of pyrazole endowed benzimidazole hydrazones (**28a-f**)



X = O; R_1 = H, 5-CH_3, 5-NO_2, 5-Cl

X = S; R_1 = H, 3-CH_3

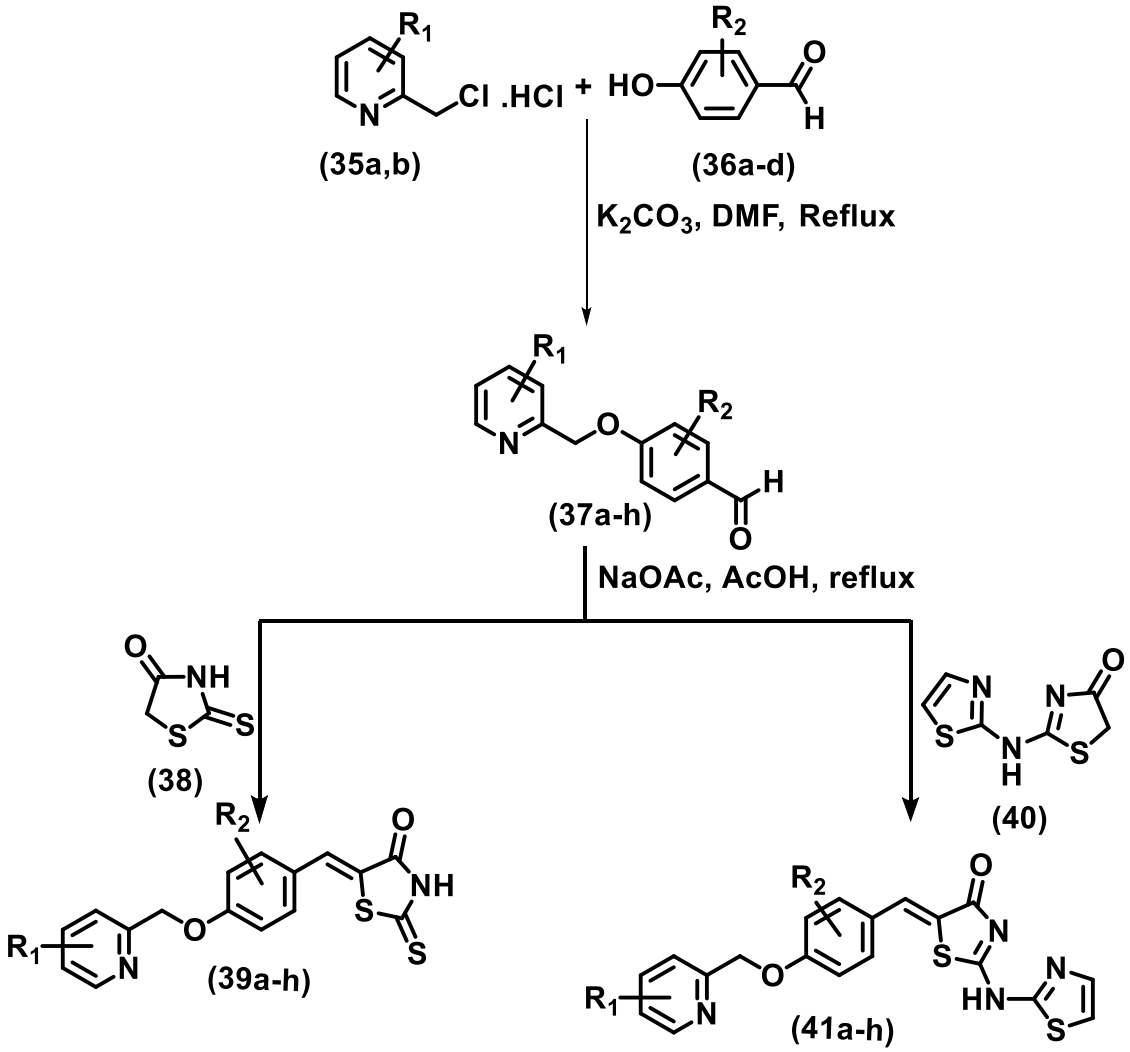
Scheme 4.2: Synthesis of pyrazole endowed benzimidazole hydrazones (**34a-f)**

All the final compounds were characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopic techniques. These compounds were screened for antimicrobial and anti MRSA activity. Moreover, most potent compound was screened for time-kill kinetics experiment on *S. aureus* organism. The results of these studies are described in this chapter.

Chapter 6

Synthesis of newer pyridine endowed rhodanine and thiazolone derivatives as antibacterial agents

This chapter describes the synthesis of some novel pyridine endowed rhodanine and thiazolone derivatives to explore their antimicrobial and anticancer properties. Initially, substituted 2-chloromethylpyridine hydrochloride (**35**) in DMF were treated with various substituted hydroxy benzaldehyde (**36**) in the presence of potassium carbonate as a base at 80 ^{0}C for seven hours to obtain substituted pyridine-benzyloxy aldehydes **(37a-h)**. These products were refluxed for eight hours with rhodanine **(38)** and thiazolones **(40)** in the presence of sodium acetate in acetic acid to yield pyridine endowed thiazolidinone derivatives **(39a-h** and **41a-h)** respectively. All the compounds were characterized by FT-IR, ^{1}H NMR and mass spectroscopic techniques. These compounds were evaluated for their antimicrobial activities. Moreover, molecular docking and dynamics studies was carried out for both Gram positive and Gram negative β-lactamase to understand its mode of action. The results of these studies are described in this chapter.



R_1 = 3,4-$(OCH_3)_2$, 4-OCH_3 CF_3-3-CH_3; R_2 = H, 2-Br, 3-Br, 3-OCH_3.

Scheme 5: Synthesis of pyridine endowed rhodanine and thiazolone derivatives **(39a-h** and **41a-h)**

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List of Publications in Journals

1. *In vitro* and *in silico* studies of fluorinated 2, 3-disubstituted thiazolidinone-pyrazoles as potential α-amylase inhibitors and antioxidant agents, **Ganavi D,** Ramu R, Kumar V, Patil S M, Martiz R M, Shirahatti P S, Sathyanarayana R, Poojary B, Holla B S, Poojary V, Kumari K N., Archiv der Pharmazie, 2022; 355(3): 2100342. https://doi.org/10.1002/ardp.202100342
2. Pyrazole-Imidazopyridine Hydrazones: Synthesis, α-Glucosidase, α-Amylase Inhibitory Activity and Computational Studies, **Ganavi D,** Patil S M, Kumar V, Ramu R, Poojary B. ChemistrySelect. 2023; 8(30): e202300778. https://doi.org/10.1002/slct.202300778

Conference Proceedings

1. An efficient one-pot synthesis of 1,2-substitited benzimidazole-5-carboxylates as anticancer agents and molecular docking studies, **Ganavi D,** Vasantha Kumar, Ashwini Prabhu, Ramith Ramu, Boja Poojary, presented at International Conference on Chemical Science -ICCS-2023 organized by Dept of Science, Christ Acadamy Institute for Advanced Studies, Bangalore on June 22nd and 23rd, 2023.
2. Imidazopyridine linked pyrazole derivatives as a potential dual inhibitor of alpha-Glucosidase and alpha amylase and molecular docking studies, **Ganavi D,** Vasantha Kumar, Ramith Ramu, Boja Poojary, presented the paper at Two days International Conference on Drug discovery 2022 on November 10th and 11th, 2022.
3. Synthesis, antimicrobial and molecular docking studies of new pyrazole series of pyrazole clubbed benzimidazole hydrazone derivatives, Ganavi D., Vasantha Kumar, Vaishali, Ramith Ramu, Boja Poojary, presented the paper at Two-day National Conference on Advanced Materials for Chemical and Biological Applications at SDM College (Autonomous) Ujire, on 6th and 7th, March 2024.
4. Synthesis of newer pyridine endowed rhodanine and thiazolone derivatives as antibacterial agents, Ganavi D., Vasantha Kumar, Akhila, Ramith Ramu, Boja Poojary, presented the paper at International Conference on Transformative

Chemistry for a Sustainable Future at ST. Aloysius College Mangalore, on 15th, March 2024.

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